

APDS: Autonomous Pathogen Detection System

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February 14, 2002

U.S. Department of Energy

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Livermore
National
Laboratory

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2002 R&D 100 Awards Entry Form

APDS: Autonomous Pathogen Detection System



Richard G. Langlois, Steve Brown, Keith Burris, Bill Colston, Les Jones, Tony Makarewicz, Ray Mariella,
Don Masquelier, Mary McBride, Fred Milanovich, Shanavaz Nasarabadi,
and Kodumudi Venkateswaran

Lawrence Livermore National Laboratory

Graham Marshall, Don Olson, and Duanne Wolcott
Global FIA

An early warning system to counter bioterrorism, the Autonomous Pathogen Detection System (APDS) continuously monitors the environment for the presence of biological pathogens (e.g., anthrax) and once detected, it sounds an alarm much like a smoke detector warns of a fire. Long before September 11, 2001, this system was being developed to protect domestic venues and events including performing arts centers, mass transit systems, major sporting and entertainment events, and other high profile situations in which the public is at risk of becoming a target of bioterrorist attacks. Customizing off-the-shelf components and developing new components, a multidisciplinary team developed APDS, a stand-alone system for rapid, continuous monitoring of multiple airborne biological threat agents in the environment. The completely automated APDS samples the air, prepares fluid samples in-line, and performs two orthogonal tests: immunoassay and nucleic acid detection. When compared to competing technologies, APDS is unprecedented in terms of flexibility and system performance.

2002 R&D 100 Awards Entry Form

- 1. Submitting Organization:** Lawrence Livermore National Laboratory
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Zip/Postal: 94551
Country: USA
Submitter's Name: Richard G. Langlois
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Fax: (925) 422-2282
Email: langlois1@llnl.gov

AFFIRMATION: I affirm that all information submitted as a part of, or supplemental to, this entry is a fair and accurate representation of this product.

Submitter's signature: Richard G. Langlois

2. Joint entry with (company names).

(If necessary, list additional companies on a separate sheet and check here:)

Organization name:

Address:

City:

State:

Zip/Postal:

Country:

Contact Name:

Phone:

Fax:

Email:

3. Product name: Autonomous Pathogen Detection System

4. Briefly describe (25 words or less) what the entry is (e.g. balance, camera, nuclear assay, etc.)

An early warning system to counter bioterrorism, this self-contained, automated instrument continuously monitors air, tests for multiple pathogens, confirms positives, and reports results remotely.

5. When was this product first marketed or available for order? (Must have been first available in 2001.)

APDS was first available for licensing and listed in the Commercial Business Daily in December 2001.

6. Inventor or Principal Developer (List all developers from all companies)

Developer Name: Richard G. Langlois
Position: Senior Biomedical Scientist
Organization: Lawrence Livermore National Laboratory
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Lawrence Livermore National Laboratory

Graham Marshall, Don Olson, and Duanne Wolcott
Global FIA

7. Product price

During the past three years, we have developed a series of APDS prototypes, each having additional functional features. The development cost of this system over the three-year period was \$5M. Given expected volume savings on components and fabrication, we expect initial unit prices in the range of \$50K.

- 8. Do you hold any patents on this product?** Yes ☒ No ☐
Do you have any patents pending? Yes ☒ No ☐
Do others hold patents on this product or a similar product line? Yes ☐ No ☒

One patent has been issued regarding the APDS: U.S. Patent No. 5,589,136-Silicon-Based Sleeve Devices for Chemical Reactions; M. Allen Northrup, Raymond P. Mariella, Anthony V. Carrano, Joseph W. Balch; issued 12/31/96. (IL-9707A).

Five other patents on this system are pending.

9. Primary Function

The autonomous pathogen detection system (APDS) counters bioterrorism and contains the following features: rapid, selective collection of bioaerosols; autonomous sample preparation; multiplex immunoassay detection; and, DNA confirmation with flow-through polymerase chain reaction (PCR) technology.

Rapid, selective collection of bioaerosols — One of the easiest methods of rapidly exposing a large population to a biowarfare agent is through an aerosol (witness the effect of the recent, relatively small-scale anthrax mailroom releases). Current aerosol collectors contain dry, matrix type filters that are difficult to couple to autonomous systems, are relatively unselective in the types and sizes of particles collected, and simply do not collect enough particles over a given period to produce a sensitive enough detection capability. At LLNL, we have designed a two-stage aerosol collector that utilizes an LLNL-designed virtual impactor pre-concentration stage in front of a commercial wetted wall cyclone collector (Research International SASS 2000). The virtual impactor captures particles 1-10 μm which is the size of particles most likely to be captured in the human lung. Particles are collected in a fluid, making downstream processing much easier. The fans and inputs to the SASS 2000 have all been replaced to obtain much higher collection rates, up to 3000 liters of air per minute flow through the detection system, allowing many more particles to be collected over a shorter period (without the enhancements the collection rate was only 200 liters per minute). The enhancements also improve the system sensitivity and reduce the collection times. An on-board computer controls air flow rates and the size range of particles collected, while a commercial particle counter provides real-time feedback on the size and quantity of particles collected.

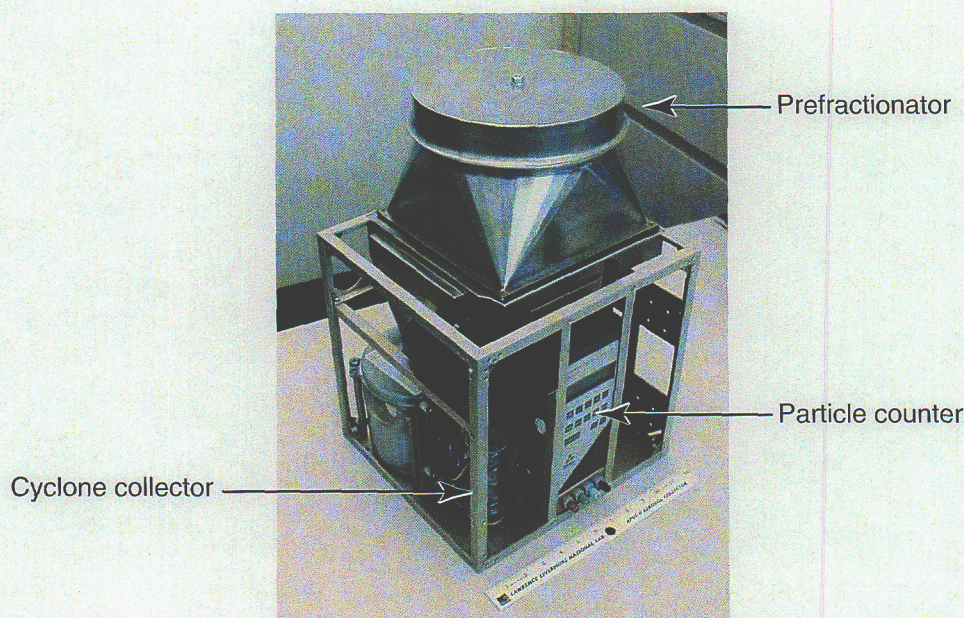


Figure 1. APDS aerosol collector combines a prefractionator with a high throughput liquid sampler for efficient, rapid bioaerosol collection.

Autonomous sample preparation — Our sample preparation module reproduces the function performed by biologists who routinely perform “wet” chemistry on the bench: moving the sample from the aerosol collector, preparing the sample (mixing, filtering, incubation, etc.), and delivering the sample reaction volume to the immunoassay and nucleic acid detectors. Conventional sample preparation instrumentation used, for example, in high-throughput drug discovery analyses, use robotic manipulation of micropipettes coupled to disposable filter wells. Since robotics are inherently complex (and difficult to scale) we chose a powerful, highly flexible technique called sequential injection analysis (SIA) as the basis for our sample preparation module. Global FIA (Gig Harbor, Washington) developed SIA and they have been consulting with LLNL on this technology and testing components for potential use. Automation is achieved through the manipulation of small solution zones under conditions of controlled dispersion in narrow bore tubing. SIA makes use of a multi-position selection valve and a syringe pump to construct a stack of well-defined sample and reagent zones in a holding coil of narrow bore tubing. By appropriate manipulation of this zone stack, a wide range of sample handling unit operations can be accommodated. The pump is used to move the sample from one device to the next achieving the required sample manipulation in the process. Once a detectable species has been formed, the zone stack is transported to the immunoassay and nucleic acid detectors.

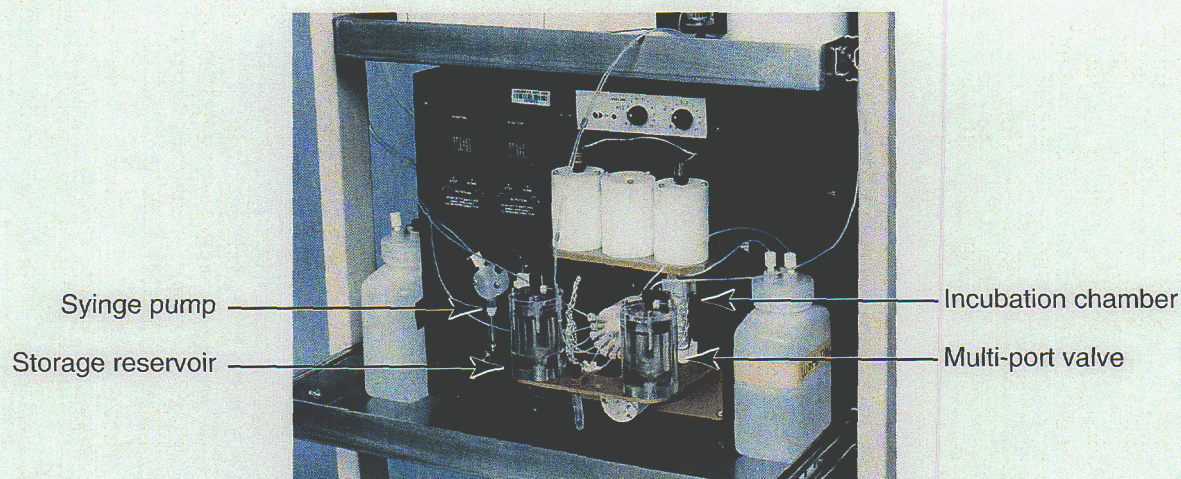


Figure 2. APDS sample preparation unit uses simple SIA hardware (syringe pump, holding coil, multi-port valves, incubation chamber) to reproduce lab bench protocols.

Multiplex immunoassay detection — The heart of our detection capability is the use of “liquid arrays”, a novel, highly multiplexed assay that competes (in bead format) with “computer chip” platforms. Luminex Corporation (Austin, Texas) developed the most robust platform for these new microbead-based assays, the Lx100. Collaborating with Luminex, LLNL is adapting this technology to detect pathogens and modifying their software to meet these new needs. The detection principle is built around the use of optically encoded microbeads that can be used as assay templates (Fig. 3). Small diameter polystyrene beads are coded with 1000s of antibodies. The sample is first exposed to the beads and the bioagent, if present, is

bound to the bead. A second, fluorescently labeled antibody is then added to the sample resulting in a highly fluorescent target for flow analysis. Since the assay is performed on a microbead matrix, it is possible to measure all types of pathogens, including viruses and toxins. Each microbead is colored with a unique combination of red and orange emitting dyes. The number of agents that can be detected from a single sample is limited only by the number of colored bead sets.

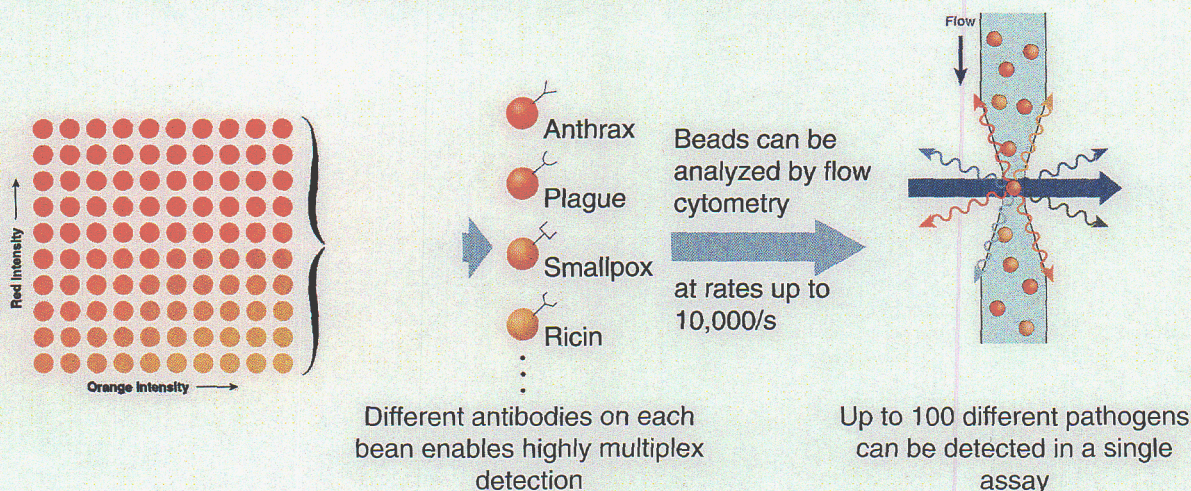


Figure 3. Liquid arrays of optically encoded microbeads allow highly multiplexed measurements to be made of any bioagent type.

Luminex has developed their a bead staining technique with sufficient precision to make a 10 x 10 array of beads, making 100-plex bioagent detection viable. We have currently demonstrated the use of this technology for measuring a wide range of bioagents at sensitivities and selectivities comparable to non-automated conventional immunoassay techniques (such as enzyme-linked immunosorbent assays) that take 4-6 times as long. A novel component of this system is the use of additional bead types used as internal positive and negative controls to monitor each step in the sample preparation process. This imparts a measure of quality control noticeably lacking in most other approaches, and imperative in an autonomous system. Sample preparation followed by LX-100 analysis can be completed within 25 minutes with the APDS fluidic system. Results of a 24-hour autonomous operation are shown in Figure 4.

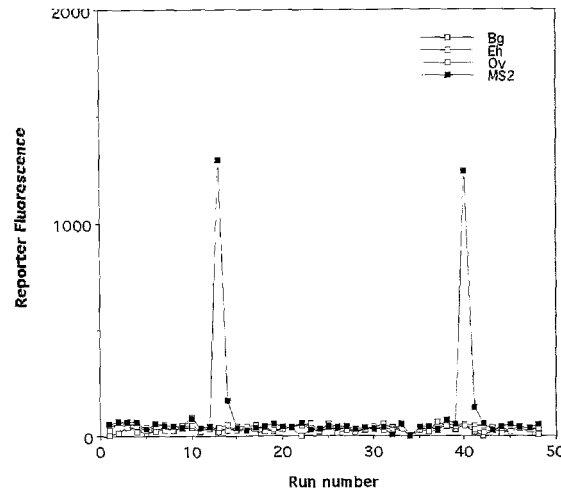


Figure 4. This example of APDS operation shows that samples were collected every half hour and analyzed for the presence of four bioagent simulants: *Bacillus globigii* (Bg), an anthrax surrogate; *Erwinia herbicola* (Eh), a bacterium; ovalbumin (Ov), a toxin surrogate; and Bacteriophage MS2, a virus. Samples of the virus surrogate (MS2) were added and detected (green spikes) at two time points in the trial.

DNA confirmation with flow-through PCR technology — The APDS contains a second detection system that is based on nucleic acid amplification and detection. An archived sample is mixed with the TaqMan reagent and introduced by the SIA technique into the flow-through polymerase chain reaction (PCR) system. Specific nucleic acid signatures associated with the targeted bioagent are amplified up to a billion-fold and detected as a fluorescence change from a TaqMan probe. The addition of this flow-through PCR component provides a complementary detection technology to the multiplex bead assay significantly increasing system reliability and minimizing the possibility of false positives. This is particularly important for Homeland Defense applications, where the impact of evacuating a major event or office building is significant.

This prototype flow-through PCR module consists of an LLNL-designed, silicon-machined, thermocycler that is mounted in-line with our sample preparation unit (Fig. 5). The use of silicon components allows thermocycling to proceed very rapidly (less than 1 minute per heat/cool cycle). The thermocycler contains appropriate light sources and detectors to perform real-time TaqMan assays. The APDS system automatically identifies a positive immunoassay result, and then calls up the PCR analysis for confirmation.

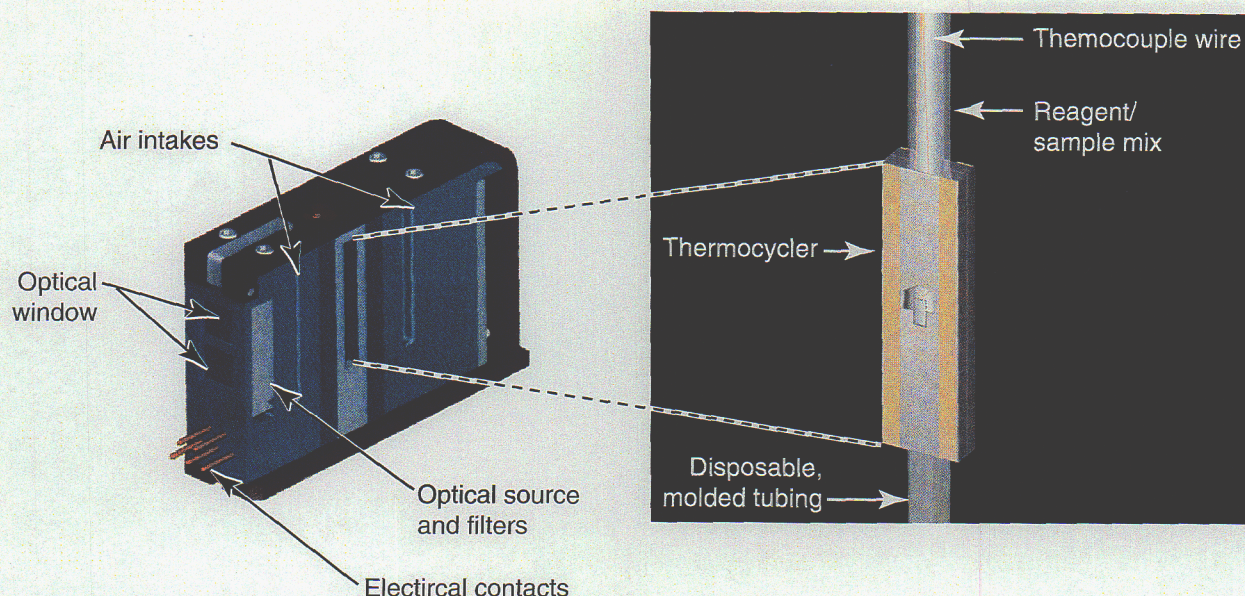


Figure 5. The flow-through PCR module contains a silicon thermocycler with a disposable, molded tubing insert. Real time temperature feedback is provided by a thermocouple trapped in the wall of the insert. Optical measurements are made through small, optical windows in the side of the silicon chamber.

10a. Competing products

There are no fully integrated systems like APDS that are commercially available in the civilian market. However, there are several military monitoring systems that share several features with this invention. Military systems currently deployed include the following: Biological Integrated Detection System with Pre-Planned Product Improvement (BIDS P3I); Interim Biological Agent Detector (IBAD); Air Base/Port Biological Detection System (Portal Shield); and, Joint Biological Point Detection System (JBPDS) which is in the initial test and evaluation stage. While specific information on performance, sensitivity, and cost are not available in the open literature, the following table provides a list of these systems' features that can be compared with APDS.

10b. Comparison to existing technology

Feature	APDS	Military Monitoring Systems			
		BIDS P3I	IBAD	Portal Shield	JBPDS
Size	2' x 2' x 5'	Modified Vehicle	4' x 3' x 6'	2' x 2' x 4' per unit	2' x 6' x 6'
Component	Self-contained Unit	Jeep & Trailer	Collector Unit & Test Lab	Multiple Units	Self- contained Unit
Current Targets	4	8	4	8	8
Potential Targets ^a	100	8	8	8	8
ID Mode(s)	2 Antibody & DNA	1 Antibody	1 Antibody	1 Antibody	1 Antibody
Fully Automated ID	Yes	No	No	Yes	Yes
Continuous Monitoring	Yes	No	No	No	No
Quantify agent concentration	Yes	Yes	No	No	No
Stand-alone Unit	Yes	Yes	No	No	Yes
Primary Use	Civilian, Diffuse Cloud	Battlefield, Large Cloud	Battlefield, Large Cloud	Battlefield, Large Cloud	Battlefield, Large Cloud
ID Time	30'	30'	45'	25'	15'
Single-use Disposables	No	Yes	Yes	Yes	Yes
Fully Automated Confirmation with 2nd ID Mode	Yes	No	No	No	No

a. Possible with existing hardware

☐ Unique to APDS system

10c. Improvement on competitive technologies

- **Measures up to 100 different agents per sample.** The ability to detect and analyze large numbers of agents is critical in the area of counter-terrorism given the large number of pathogens potentially available to terrorists. Our use of flow cytometric analysis of color-coded beads provides a substantially higher level of

multiplexing compared with competing systems. This approach also provides the flexibility to easily integrate new bead-based assays for additional agents and assays with improved performance.

- **Continuously monitors with a fully automated system.** Continuous monitoring provides the ability to detect low concentrations of bioagents that would not be detectable if the system were triggered by a change overall aerosol particle count. Our use of automation is critical to reduce the substantial cost and manpower of manual analysis of 50 samples per day per machine.
- **Identifies pathogens using two orthogonal detection methodologies.** Minimizing the possibility of false alarms is critical for an unattended monitoring system. Our use of two identification technologies provides confirmatory data on a suspected event increasing the confidence in agent identification compared with competing systems that use only one technology. The probability of false alarms with APDS is also greatly reduced if coincident identifications from two different tests are required before action is taken.

11a. Principal applications of this product

APDS monitors the environment to protect the public from the release of hazardous biological agents. While the primary focus has been on protection of civilians from terrorist attacks, the same system could also have a role in protecting military personnel from biological warfare attacks.

APDS is a countermeasure to bioterrorism, one of the most serious threats to the safety of U.S. citizens. The current interest in biological terrorism has its roots in the conflict between the U.S. and Iraq in the early 1990s when it was found that our armed forces were inadequately prepared to defend themselves from potential biological-based aggression from Iraq. This prompted a major Department of Defense (DOD) effort to produce more effective means to detect biological pathogens and protect the soldiers.

By the mid-1990s, the U.S. Congress began to assess the vulnerability of the U.S. civilian population to biological terrorism and found us considerably lacking in our ability to cope with even a small-scale biological event. Initial thinking was that the DOD technology could be readily transferred to the civilian arena. However, upon further reflection, it was concluded that although there was overlap between military and civilian defense needs, in the case of a biological threat, there are marked differences: (1) the soldier is trained and equipped with protective gear so he may respond to a threat quickly enough to prevent a lethal dose; (2) military intelligence usually reduces the potential threat to a relatively small number of biological agents; and, (3) military battlefield tactics are designed to minimize the density of soldiers. The civilian population, however, is neither trained nor equipped, is vulnerable to any conceivable pathogen and often gathers in large crowds (special events, sporting venues, etc.) where a small release could potentially infect thousands. In response to these differences, federal agencies, including DOE, have recently begun funding directed research efforts to reduce civilian biological terrorist vulnerabilities. A near

term goal of these efforts is an integrated network of sensors and analytical software that will help us protect critical assets such as subway systems, or major events such as the 2002 Winter Olympics.

The Lawrence Livermore National Laboratory (LLNL) is a major participant in the above efforts and, as a result, has intimate knowledge as to the performance parameters of all requisite technologies and techniques that can be applied to civilian, biological, counter-terrorism. At present there are more than 30 pathogens and toxins on various agency threat lists. Public health personnel rarely see most of the pathogens so they have difficulty identifying them quickly. In addition, many pathogenic infections aren't immediately symptomatic, with delays as long as several days, limiting options to control the disease and treat the patients. The lack of a practical monitoring network capable of rapidly detecting and identifying multiple pathogens or toxins on current threat lists translates into a major deficiency in our ability to counter biological terrorism. APDS addresses that deficiency.

APDS instruments can be used to implement a number of different monitoring applications. Single units could be transported to venues of high profile events for short-time, intensive monitoring. A political convention, for example, might require hourly measurements for a period of one week. An alternative approach would be permanent installation in a major public building or commercial center. In this application, measurement intervals might be increased to 8-hours to provide full coverage with less time resolution to conserve reagents. Finally, the full benefits of autonomous monitoring units will be achieved when large numbers of units are deployed in major buildings throughout a city. All of these units can be networked to a single command center so that a small group of technical experts could maintain and respond to alarms at any of the networked sensors.

11b. Other applications

The bead-based immunoassay and flow through PCR capabilities of APDS can also be used to monitor a variety of other environmental or clinical pathogens. Mobile units could be transported to suspected "sick buildings" to test for mold or fungal spores that might be causing tenant illnesses. Units with reagents for animal diseases could be placed in livestock transport centers or feedlots to rapidly detect airborne pathogens and protect against disease outbreaks. Finally, monitors in hospitals could be used to test for airborne spread of contagious materials among patients.

12. Summary

APDS can protect civilian populations from bioagents by monitoring the environment continuously, accurately, quickly, and automatically. This system provides a totally new capability to detect and identify covert releases of biowarfare agents. The key to this approach is to develop flow-through sample processing and detection technologies. APDS uses simple fluid movements to automatically perform large numbers of sequential analyses. Use of this invention for environmental monitoring to counter biological terrorism would benefit public health with rapid detection and treatment after a biological event, as well as deter terrorist use of biological agents. Manual sample collection and laboratory analysis are an option, but are much slower, more labor intensive (require 6 to 10 people whereas APDS requires 1 to 2), and require extensive laboratory resources.

ORGANIZATION DATA

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Time Magazine 11/26/01

[Security]

INNOvators
TIME 100: THE NEXT WAVE

Making the World Safer

[THE GERM DETECTOR]

Sniffing Out Bioterrorism

Lots of little boys ask Santa for a bike or a baseball bat. But when Richard Langlois was growing up in El Cerrito, Calif., all he wanted for Christmas were the test tubes and beakers pictured in his laboratory-supply catalogs.

These days, Langlois' equipment is supplied by Lawrence Livermore National Laboratories, where the biologist has been working for two years on a piece of equipment that is suddenly commanding great interest: a continuous air-monitoring system that can detect within an hour the presence of any bacteria or virus in a basketball stadium, shopping mall or other indoor place. "It's like a smoke alarm" for



harmful biological agents, says Langlois.

The benefits of Langlois' Autonomous Pathogen Detection System are obvious. Instead of waiting for someone to come down with anthrax or smallpox—or running a blood test on folks who think they might be infected—the APDS might give public-

RICHARD LANGLOIS

The biologist, 54, is developing a device that can detect any of 100 different pathogens in an hour—before anyone gets sick

safety officials sufficient warning to evacuate the area before anyone got exposed. In a world where bioterrorism is no longer un-

thinkable, the APDS could serve as the first line of defense.

The system works by sucking in an air sample, analyzing its components, then putting out a report at fixed intervals, up to 48 times a day. At its core is a flow cytometer—or cell sorter—that Langlois co-

invented in the late 1970s. The device shines laser beams on chromosomes within cells to make a quick genetic ID. A spin-off of early research into mapping the human genome, the cell sorter is now a standard tool for diagnosing AIDS, leukemia and other cancers. Langlois even took it to Chernobyl to assess workers' genetic damage from radiation exposure after the 1986 nuclear reactor accident.

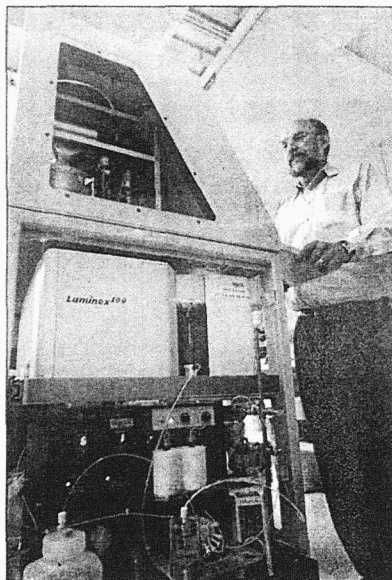
While other air sniffers are in the works or already in use, the refrigerator-size APDS stands out for its ability to rapidly detect even trace amounts of 100 different germs. To avoid the nuisance of false positives, suspected pathogens undergo a second, DNA-based test before officials are alerted.

Using the technology to sniff for biological weapons makes sense. The system was not supposed to be commercially available for two years, but the post-Sept. 11 sense of urgency could help speed development. "As I see people dying from anthrax," says Langlois, "it motivates me to work extra hard." —By Anita Hamilton

Excerpts from media reports

L.A. Times 10/22/01

LAB: Research Promises Faster Response to Biological Attacks



ROBERT DURELL / Los Angeles Times

Livermore lab's Richard Langlois demonstrates the Autonomous Pathogen Detection System, the biological-agent equivalent of a smoke detector that constantly monitors the air for pathogens.

"Civil defense: Experts at the Livermore lab adapt their experience building the U.S. nuclear arsenal to develop defenses for the likes of anthrax.

...scientists plan to deploy a second device that could constantly monitor and analyze the air in the convention hall, subway or even an entire city – and send out an alarm if pathogens reach a trigger level.

Already, following the Sept. 11 attacks, Lawrence officials have installed a less advanced system of biocollectors in one undisclosed city

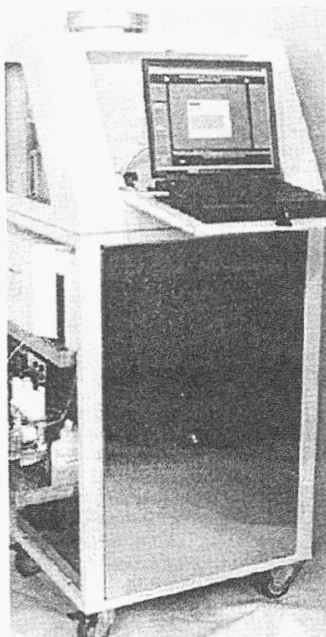
...The Autonomous Pathogen Detection System is probably a year or two from production, officials say.

The prototype – with an air intake and computer monitor atop a maze of wires, tubes and chemical tanks — sucks in air, then collects particles in a liquid for analysis. The results appear on a computer screen every 30 minutes..."This device could give an early warning and save many lives."

To protect a complex of buildings or a city, a network of the devices could be linked to an emergency response center, researchers say.

THE TECHNOLOGY

Developing an Early Warning System for a Biological Attack Proves Difficult



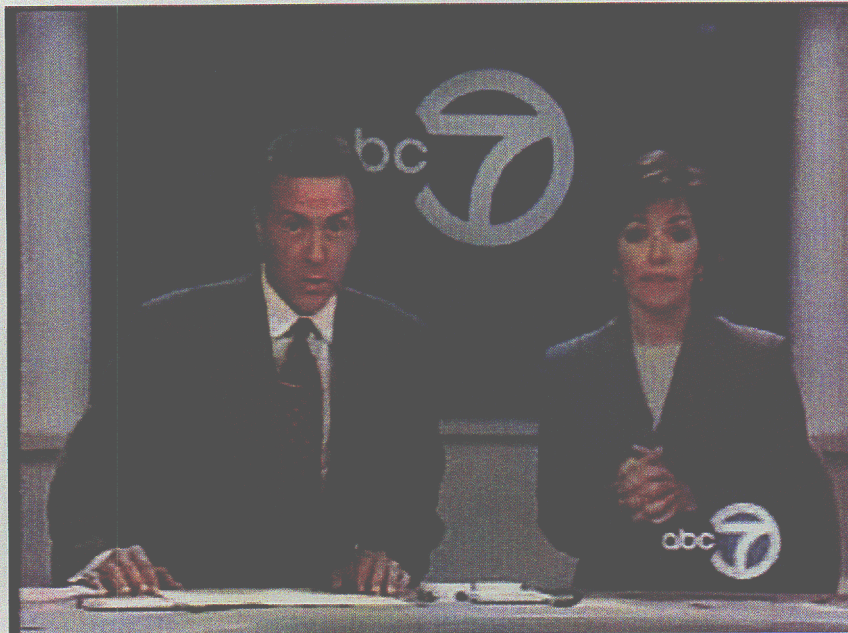
The Autonomous Pathogen Detection System can identify multiple airborne biological agents.

The fact that people infected with anthrax are likely to survive if they are promptly treated has reinforced the broad agreement among scientists that the best defense against a biological attack is an early warning system. But designing and building such a system had proved difficult. Current detection systems, like the devices put around the Pentagon, are bulky, expensive, slow to discern problems and prone to false warnings...

The Department of Energy is trying to use off-the-shelf technology to build a system for use in civilian areas like airports, stadiums and subways...

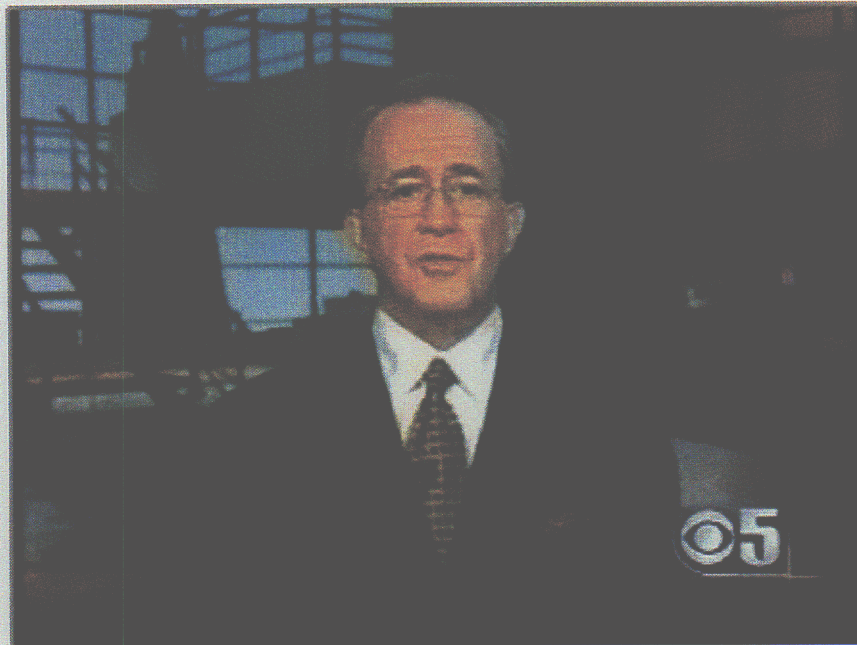
The next generation system, being developed at Lawrence Livermore National Laboratory, would be autonomous. The system draws in air and traps the particles in a liquid, which then flows past tiny beads coated with antibodies that hook onto only certain pathogens. A second genetic test would confirm results.

KGO TV News (ABC San Francisco) 9/25/01

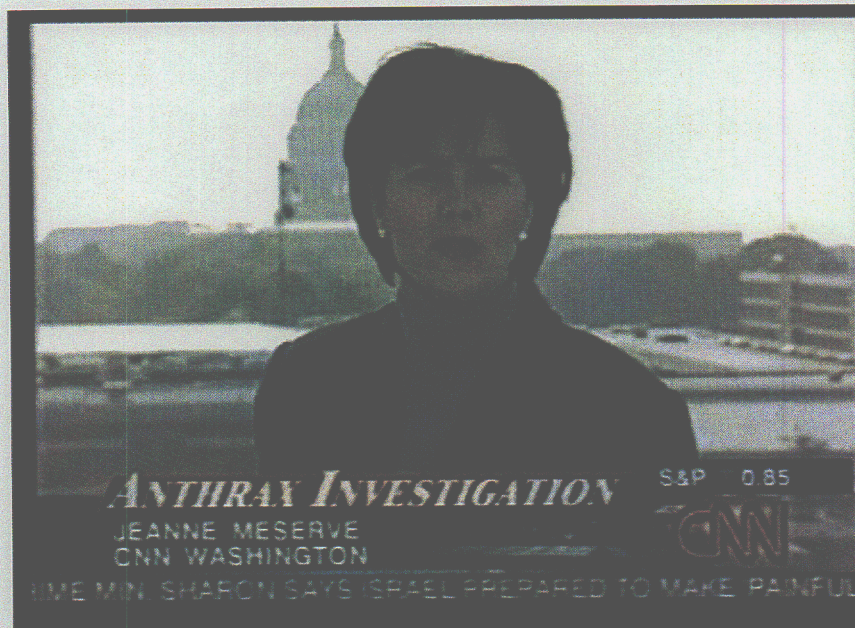


“The attacks of September 11th have obviously raised fears of continued terrorism, maybe even a viological attack next time. The Livermore Laboratories have come up with a new line of defense to fight terrorist. APDS is LLNL’s latest weapon against bioterrorism: a machine that sucks in air, capturing the particles and testing them for dangerous biological agents, including anthrax and bubonic plague. A computer records the measurements every half hour... The devices could monitor locations that might be targets for bioterrorist attacks, an airport or a subway station...”

KPIX TV News (CBS San Francisco) 11/1/01



“New Technology being developed here in the Bay Area could provide an early warning system and potentially save thousands of lives... Researchers at the Lawrence Livermore National Laboratory have been working to create a kind of smoke detector for biological weapons, the Autonomous Pathogen Detection System (APDS)...We want something that will sit there in an unattended way and monitor continuously, day after day. That’s what a smoke alarm does. The problem is that it’s a lot harder to do that with biological agents than it is for smoke. But the APDS can recognize some of the most dangerous substances in the biological arsenal. First, air samples are dissolved in liquid that passes over thousands of tiny colored beads coated with the antibodies of different pathogens like anthrax. If the pathogens are present, those antibody beads will bind to them. A laser then illuminates the beads and reads the color to determine which germs are present... Researchers from LLNL are also members of a mobile response team. It was formed to analyze large areas for biological or chemical contamination...”



“One of the most worrisome aspects of anthrax or terrorism’s other biological threats is certainly the fact that victims don’t know they’ve been exposed until the danger had taken root... How do we detect a biological attack on civilians? A huge push is on in government labs and universities and in private industry to use DNA technology in the field to identify anthrax, small pox, and a wide range of other biological agents, quickly and relatively cheaply... This is the APDS. Within a year or two, detectors like this might be deployed. It’s a fully automated system that behaves like a biological smoke alarm. It continuously monitors the air, pulling out particles for DNA analysis, identifying pathogens. Someday there might be networks of sensors providing protection for metropolitan areas.”